TASK 10. EFFICACY AND TOXICITY OF TREATMENTS FOR BACTERIAL KIDNEY DISEASE IN CHINOOK SALMON (PROGRESS REPORT: 1 JUNE 1999 THROUGH 31 MAY 2000)

by

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Introduction

Bacterial kidney disease (BKD) is the major infectious disease preventing successful culture of salmonids in the Pacific Northwest. Between 1993-94 this disease was responsible for catastrophic losses of endangered Redfish Lake sockeye salmon held as captive broodstock. Long-term prophylactic administration of erythromycin has become common in recent years to reduce losses due to BKD. Results of preliminary studies with Lake Wenatchee sockeye salmon suggest that erythromycin may have a negative effect on gamete viability. Recent experiences at Manchester Research Station with Catherine Creek, Lostine River, and Lemhi River spring chinook salmon have shown currently mandated treatment regimens may elicit fatal toxicity reactions. Until alternative therapeutics, such as azithromycin, are identified and proven efficacious for treating BKD in captive broodstock, or until an effective vaccine becomes available, use of erythromycin will continue.

First, the efficacy of erythromycin and azithromycin in treating BKD will be investigated in a BKD challenge experiment. Second, in order to design treatment regimens which avoid toxic reactions and reductions in reproductive success attributable to erythromycin, it is first necessary to identify the cause of each toxic reaction and the condition under which it appears. This experiment will be carried out in large-scale rearing studies, together with supporting laboratory analyses.

BKD-caused epizootics continue to impact captive rearing programs of both sockeye and chinook salmon significantly (Schiewe et al. 1997). There is no vaccine available to protect salmon from infections with *Renibacterium salmoninarum*, the causative bacterium of BKD. Erythromycin has been the primary antibiotic used by fish

culturists in an attempt to prevent and control *R. salmoninarum* (Elliott et al. 1989), administered orally through feed or by injection of maturing adults. However, while use of erythromycin usually results in short-term health improvement of infected fish, it fails to eliminate the infection completely, and symptoms of disease often return after treatment ends.

Azithromycin is a promising candidate for use as a therapeutic and prophylactic agent for BKD in captive broodstock salmon. This new macrolide antibiotic concentrates in polymorphonuclear leukocytes, macrophages and fibrocytes (Peters et al. 1992). *R. salmoninarum* has been shown to invade these cell types, which in turn protect the organism from the host humoral immune system (Bandin et al. 1993; Gutenberger et al. 1997). Azithromycin has strong bactericidal activity against *R. salmoninarum in vitro* and has shown efficacy *in vivo* in prior experimental BKD infections of salmonids.

There are two principal experiments in the work plan: A) compare the efficacy of azithromycin and erythromycin in the treatment of acute BKD in fall chinook salmon; and, B) evaluate the long-term prophylactic use of azithromycin and erythromycin in the development of acute toxicity syndrome and effects on gamete development and quality. The progress of each are reported separately below.

Experiment A

The main objective is to determine the relative efficacies of the two macrolide antibiotics, erythromycin and azithromycin, in the treatment of acute BKD, that is, when disease symptoms first appear. To carry this out, test fish were infected by intraperitoneal injection of a virulent strain of *R. salmoninarum.* This progress report includes efforts from the 1998-1999 and 1999-2000 work plan under this subtask.

Experiment B

The three main objectives are as follows:

(i) Determine how captive fall chinook salmon broodstock gonad development, gamete viability, and survival of the progeny though the swim-up stage are affected by long-term prophylactic administration of erythromycin or azithromycin.

(ii) If the experimental fish are found to be naturally infected with *R. salmoninarum,* the effects of two erythromycin treatment regimens (two or four treatments annually) on BKD incidence will be measured from the first feeding fry through mature adult life history stages.

(iii) Determine whether erythromycin toxicity responses are related to treatment frequency. This aspect of the study will include a measurement of residual tissue concentrations and will document underlying tissue damage associated with the syndrome. While the study focuses on erythromycin as it is the current antibiotic of choice and the one apparently causing the severe toxic side effects, the long-term effects of azithromycin treatment will also be assessed concurrently.

Work completed

Experiment A

In both years, approximately 2,000 Minter Creek fall chinook salmon were selected for the study. The one major difference between 1998 and 1999 studies was that the fish in the latter study were pre-vaccinated against *Vibrio* after smolting (Alpharma Alpha Dip 2100 *Vibiro anguillarum-ordalii* bacterin, delivered by intraperitoneal injection). In both studies, the fish were acclimated in pathogen-free seawater at the Manchester Research Station for 4 wk prior to *R. salmoninarum* challenge. Fish were fed a standard unmedicated diet. During adaptation, randomly selected fish were screened for the presence of BKD by ELISA and FAT of kidney tissues, and by RT-PCR of blood samples (Rhodes et. al. 1998). Prior year pre-screening showed that ~25% of the fish were infected with *R. salmoninarum* in the absence of clinical signs of disease.

In both years challenge studies included two groups of fish. The group designated for 'challenge' were inoculated intraperitoneally (IP) with a dose of *R. salmoninarum* (ATCC strain 33209) designed to produce clinical acute BKD (approximately 1 x 10^6 bacteria/12 g fish). In both years this method successfully induced fulminating BKD in the study chinook salmon with significant mortality starting 3-wk post-injection. The group designated 'unchallenged' were inoculated IP with an equivalent volume of phosphate buffered saline to duplicate the stress of handling and inoculation of the challenged fish. This group also served as a negative control for any deleterious effects of subsequent antibiotic treatment, activation of quiescent pre-existing BKD, or the appearance of other unrelated disease and mortality.

After inoculation the fish were randomized and transferred into tanks (1.6 m d), with each tank containing 75 fish (1998) or 100 fish (1999). Ten days post challenge (a time period when first mortalities from this challenge usually occur), fish were switched to a feed with antimicrobial supplement, as follows:

1998

Duplicate groups of *R. salmoninarum*-challenged and unchallenged fish were fed:

- BioDiet + no medication,
- BioDiet + erythromycin (dosage to equal 100mg/kg/d for 28 d; standard INAD treatment protocol),
- BioDiet + azithromycin (10 mg/kg/d for 14 d).
- BioDiet + azithromycin (30 mg/kg/d for 14 d).

1999

Triplicate groups of *R. salmoninarum*-challenged fish were fed:

- BioDiet + no medication,
- BioDiet + erythromycin (dosage to equal 100mg/kg/d for 28 d; standard INAD treatment protocol),
- BioDiet + azithromycin (30 mg/kg/d for 14 d).

As in the 1998 study, groups of unchallenged and challenged fish were fed identical diets but, due to tank number limitations, unchallenged/unmedicated and unchallenged/erythromycin groups were carried out in duplicate only. The health of the fish were monitored, and all mortalities necropsied to verify death by BKD. Kidney tissues were examined by FAT to determine levels of *R. salmoninarum* (more sensitive ELISA and RT-PCR assays were to be performed on fish with inconclusive FAT). Representative fish from all treatments were routinely sampled and analyzed for the presence of the bacterium to monitor changes in bacterial load after treatment. In the 1999-2000 study, treatments were repeated on surviving fish after 12-16 wk, and the experiment continued 5 wk beyond that point in time.

Experiment B

Phase 1: First feeding to smolt -- During January 1999, approximately 3,150 George Adams fall chinook salmon were transferred to the Big Beef Creek Hatchery. Prior to initiation of exogenous feeding, 400 fish were randomly stocked into each of 14 isolation tanks (two tanks per treatment). Using this experimental design, the following feedings were carried out:

- No treatment.
- Erythromycin administered orally at a rate of 100 mg/kg/d for 28 d, or azithromycin administered orally at a rate of 30 mg/kg/d for 14 d, the first treatment administered at initiation of exogenous feeding, the second during sexual differentiation (2 g average wt), and the third just prior to smoltification (ca. 7 g average wt).
- Erythromycin administered orally at a rate of 100 mg/kg/d for 28 d, or azithromycin administered orally at a rate of 30 mg/kg/d for 14 d, the first treatment administered during sexual differentiation (2 g average weight) and the second just prior to smoltification (ca. 7 g average weight).
- Erythromycin administered orally at a rate of 100 mg/kg/d for 28 d, or azithromycin administered orally at a rate of 30 mg/kg/d for 14 d, with the only treatment administered just prior to smoltification (ca. 7 g average weight).

At the beginning of the trial and following each treatment, fish from each experimental tank were collected for measurement of tissue antibiotic concentrations, and for histological evaluation of brain, heart, kidney, liver, spleen, stomach, pyloric cecae, and intestine for abnormalities associated with erythromycin or azithromycin toxicities. The collected specimens are currently being analyzed following methods described in the work plan.

Phase 2: Smolt to maturity -- Phase 2 of the study was initiated in June 1999. One hundred twenty fish from each tank were PIT tagged and divided randomly into three groups of 40 fish each, which were combined as follows:

Tank 1: Forty fish from Treatments 1-7, replicate A

Tank 2: Forty fish from Treatments 1-7, replicate A

Tank 3: Forty fish from Treatments 1-7, replicate A

Tank 4: Forty fish from Treatments 1-7, replicate B

Tank 5: Forty fish from Treatments 1-7, replicate B

Tank 6: Forty fish from Treatments 1-7, replicate B

Two tanks of fish were then assigned to each of the experimental treatments. Using this experimental setup, the following feedings are being carried out.

- No treatment.
- Erythromycin administered orally at a rate of 100 mg/kg/d for 2 d, twice per year until mature (November-December 2001).
- Erythromycin administered orally at a rate of 100 mg/kg/d for 28 d, four times per year until mature (November-December 2001).

To date, the erythromycin treated groups have received either one (of two), or three (of four) treatments scheduled for the 1999-2000 reporting period.

Work to be Completed

Experiment A

1998-1999 study -- All samples from the study have been analyzed. Stringent statistical analysis on the data still needs to be performed before broader conclusions can be drawn. It is anticipated that all statistical analysis will be completed by 1 October 2000. In addition, *Vibrio* infections in all groups led to *Vibrio* vaccination of all fish in the 1999-2000 study.

1999-2000 -- This experiment was recently completed and laboratory analysis of samples should be finished by 1 August 2000. Data analysis and preparation of an interim report and recommendations will be completed by 31 December 2000. A final report incorporating data from both studies will be prepared during the June 2000-May 2001 reporting period.

Experiment B

Phase 1: First feeding to smolt stage -- All samples collected during this phase are currently being analyzed. All laboratory work will be completed by 1 October 2000. Data analysis and preparation of an interim report and recommendations will be completed by 31 December 2000.

Phase 2: Smolt to maturity -- Samples will be collected in September 2000 and September 2001 to evaluate the effects of the various treatment regimens (i.e., pre- and post-smolt treatments) on BKD prevalence, drug clearance rates, and organ histology. Maturing females will be spawned towards the end of 2001 to evaluate the effects of the various treatment regimens on reproductive success. Laboratory work will be completed by June 2001. The final report will be prepared during the June 2001-May 2002 reporting period.